OOGENESIS IN HABROTROCHA TRIDENS (MILNE)

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The bdelloid rotifers of about 200 species, classified into 19 genera and 4 families, reproduce exclusively by parthenogenesis, males being unknown in this group. It is therefore interesting to study the behavior of their chromosomes during oogenesis. I have reported such a study on one of them, *Philodina roseola* (1956). Some of the findings reported in that paper are as follows: 1. In *Philodina roseola,* there are two maturation divisions, both equational. 2. No indication of synapsis has been observed between any two of the chromosomes. Individual chromosomes even in the earliest oocytes were observed to be in a condensed state. The anaphase chromosomes of the oogonial division do not despiralize in forming the nuclei found in the syncytial ovary. The chromosomes, after the last oogonial division, remain condensed, and, by progressive packing together, they form first a ring and then a homogeneous and spherical mass of chromatin occupying the center of the nucleus. When one of these nuclei is isolated by the ovary to form an oocyte, its condensed chromosomes do not despiralize into leptotene threads, but persist in a condensed state. As the germinal vesicle increases in size they separate from each other until finally 13 condensed chromosomes can be easily counted. 3. The zygoid chromosome number in this rotifer is 13. Three of the 13 chromosomes, two dot-shaped ones and one that is appreciably longer than the rest, are morphologically distinguishable from one another and from any one of the other ten (Fig. 37). It was suggested that the chromosomes in this group of animals may have lost their homology.

The present paper reports observations made on *Habrotrocha tridens,* which belongs to the family of Habrotrochidae. For methods employed in this study reference may be made to my paper dealing with *Philodina roseola.*

Observations

As in *Philodina roseola,* the ovary and its accessory structure, the vitellarium, in *Habrotrocha tridens* are syncytial. In mature animals, the ovary consists of about 30 nuclei, with the chromosomes in each nucleus grouped so tightly together that they form a single spherical body of smooth outline. When one of the nuclei is isolated by the ovary to form an oocyte, the individual chromosomes do not go through the meiotic changes characteristic of oocytes in other animals. They simply remove themselves from each other as contracted bodies while the nucleus increases in size.

Figure 1 illustrates the condition of the chromosomes in the nucleus of a young oocyte. At this stage, it is still difficult to differentiate the individual chromosomes. But as the nucleus enlarges, the chromosomes begin to stand out clearly as condensed bodies. If the whole history of the chromosomes in the developing egg is

not studied carefully, the thread-like structures of a less basophilic character in the nuclei illustrated in Figures 2 and 3, for instance, may be erroneously interpreted as leptotene threads, and the intensely stained true chromosomes in a condensed state regarded as heterochromatic sections of thread-like chromosomes. But as the oocyte and the germinal vesicle increase in size, the true chromosomes become more and more separated from each other and more easily differentiated tinctorially from the thread-like material. The true situation can be clearly seen in Figures 2–9. The stage of maximum growth of a germinal vesicle is seen in Figure 10. At this stage the nuclear sap appears in fixed material as a fine-meshed net. Thirteen chromosomes are spread out widely apart from each other and can be most easily counted at this stage. Two of them are appreciably smaller than the others and are dot-shaped. These two, however, are not of equal size. It will be recalled that in *Philodina roseola* there are also two dot-shaped chromosomes of unequal size. But unlike *Philodina roseola,* this form does not possess among the remaining 11 chromosomes one that is conspicuously longer than the rest.

Further development from this stage is indicated by a shrinking in mass on the part of the germinal vesicle; the nucleus thus becomes reduced in size and irregular in shape (Fig. 11). But as this takes place, the nuclear sap seems to react differently to the fixative. The fine-meshed appearance no longer prevails, and threads begin to make their appearance within the nucleus (Figs. 12–16). At this stage there is a difference between the present form and *Philodina roseola.* In the latter, the nucleus keeps on decreasing in size to a much more extreme degree, and finally becomes again rounded in outline; whereas in *Habrotrocha tridens,* the nucleus stops shrinking much earlier, and I have not observed any well-rounded germinal vesicle of reduced size (Figs. 15–17). On the contrary, when the nucleus has decreased in size to the extent shown in Figure 17, it begins to break up. Figure 17a gives the condition of the same nucleus at a lower level of focus than the one at which Figure 17 was drawn. At this level of focus, the nuclear membrane shows unmistakable signs of disintegration. After the membrane is broken, the chromosomes are set free in the cytoplasm (Figs. 18 and 19). In *Philodina roseola* the chromosomes next spread out into a more or less irregular line pressed close to the wall of the oocyte. This line formation has not been observed in the present form, and Figure 19 represents the distribution of the chromosomes most frequently observed at this time of development.

At first the cytoplasm immediately surrounding the free chromosomes does not appear any different from that seen in any other area within the egg. But when the chromosomes have pulled away from the periphery of the oocyte and have become more separated from each other, they are seen to be embedded in an area of cytoplasm which appears to be more vacuolated than the rest of the cytoplasm in the developing egg (Fig. 20). This was also observed in *Philodina roseola.* Then, also as in *Philodina roseola,* it seems that under the influence of the chromosomes, a homogeneous and light-staining material is developed in which the chromosomes are embedded (Fig. 21). It is within the area occupied by this material that the spindle is developed later. There seems to be a change going on in this material, as a result of which the area formerly occupied by the homogeneous material now appears to be traversed by threads. These threads are not as taut and trim in outline as the regular spindle fibers (Fig. 22). Such stages have been frequently observed, and in some cases the fibers do give a rather close resemblance to the regular

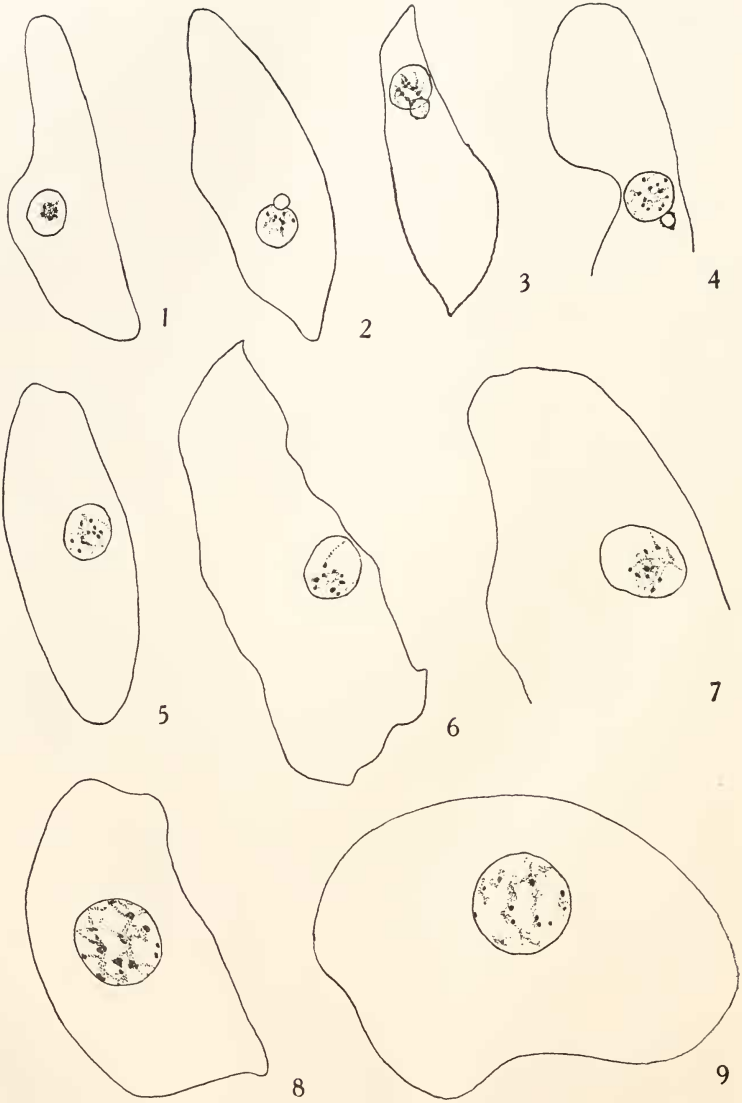


PLATE I

spindle fibers. In *Philodina roseola,* the next stage has been found to be a monopolar spindle which eventually develops into an orthrodox bipolar one (Hsu, 1956). But in *Habrotrocha tridens,* I have seen a single tripolar spindle which may be described as a compound structure formed by three different bipolar spindles, the long axes of which all lie on the same plane and so arranged with regard to each other that the structure forms a somewhat triangular configuration. The chromosomes form three separate equatorial plates, one on each component spindle (Fig. 23). Unfortunately, this is the only one I have observed in my slides.

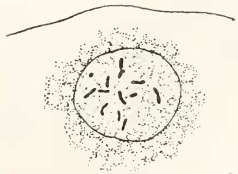
Another spindle, also the only one I have found in my material, is represented in Figure 24. There are two cones placed at an angle as shown. The chromosomes are gathered loosely at the general area toward which the truncate ends of the two cones converge. The chromosomes are not all in one level of focus. But due to the rarity of these spindles, it is simply unsafe to consider them definitely as structures normal in *Habrotrocha tridens.* They should be merely recorded and left for future discussion when more evidence becomes available. However, in view of the peculiar spindle and its manner of development observed in *Philodina roseola* where evidence was more abundant, the possibility that the two peculiar spindles observed in *Habrotrocha tridens* may express normal stages of development in this rotifer cannot be entirely excluded. If these spindles be considered as normal structures, I should then think that they represent stages following those depicted in Figures 21 and 22 and preceding that represented by Figure 25. I would assume that Figure 24 shows a stage in which the compound spindle is breaking up and a bipolar structure is in the process of forming. This process would consist of a disintegration of the base spindle in Figure 23 and a movement on the part of the chromosomes. Then a proper rotation of the two remaining cones shown in Figure 24 would produce an orthodox bipolar spindle such as that shown in Figure 25. Of course, this is largely a conjecture.

Whatever may be the true significance of these two peculiar spindles, there is no question that a bipolar spindle does finally form to effect the first maturation division in *Habrotrocha tridens.* Figures 25, 26, 27 and 28 represent lateral and polar views of the first maturation division. In Figure 29, we see two anaphase groups of chromosomes which demonstrate beautifully that this division involves no reduction in chromosomes. Figure 30 shows a polar-body and the chromosomes within the secondary oocyte being regrouped to form the metaphase plate of the second division. Figures 31 and 32 both show the metaphase spindle of the second division, each with a polar-body directly over it at a higher level of focus (Figs. 31a and 32a), which fact indicates that the long axes of the spindles of the two divisions are perpendicular to each other. The number of chromosomes which could be made out in each one of these two metaphase spindles, counting each dumb-bell shaped granule as a unit, indicates that the second division is also equational in *Habrotrocha tridens.* Usually in such cases something like 10 to 11 units could be

All figures are camera lucida drawings made at 1500 ×

PLATE I

FIGURES 1–9. Oocytes showing the condensed chromosomes in their germinal vesicles, and becoming progressively more easily distinguishable from the less intensely stained thread-like structures as the oocytes mature. In Figures 2, 3 and 4, an idiozome is shown in contact with the germinal vesicle.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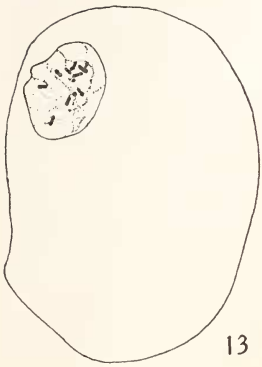
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16


17


17a


18


19


20


21

PLATE II

counted. Having seen many eggs of about this stage, I cannot help feeling that the second division takes place immediately after the first without giving the chromosomes enough time to be included within a nucleus. In *Philodina roseola,* however, a metabolic nucleus is achieved between the two divisions.

After the two maturation divisions, the comparatively large nucleus of the mature egg goes into a resting stage in which the chromosomes lose their staining intensity (Fig. 33). Figure 34 is a polar view of the metaphase plate of the first cleavage division in which 13 chromosomes with two relatively smaller ones are clearly visible. As in *Philodina roseola,* during anaphase of the first few cleavage divisions of the embryo, "elimination bodies" are visible (Fig. 35).

Discussion

In both *Philodina roseola* and *Habrotrocha tridens,* the oocyte undergoes two maturation divisions, and the zygotic chromosome number, 13, is maintained in the mature egg because both these divisions are equational. No sign of chromosome pairing has been observed. I have examined as yet too few species to venture an opinion on the question as to whether or not all the species of Bdelloidea follow this pattern of oogenesis. I can only point out the fact that the two species examined belong to two different families of Bdelloidea.

In view of the genetic principles which should apply to ameiotic parthenogenetic animals, we should not be surprised to find in their chromosomes evidence of aneuploidy, polyploidy, structural rearrangement and the loss of diploid character in both the genetic and the cytological sense. In this connection I cannot do better than to quote White (1954) (p. 341): "In ameiotic parthenogenesis genetic segregation will not occur. Recessive mutations and structural rearrangements will tend to accumulate indefinitely in such organisms, only the ones which are immediately deleterious being eliminated by natural selection. Such forms must consequently be expected to become gradually more and more heterozygous, but all the offspring of a single female will resemble their mother exactly, except for newly arisen dominant mutations and differences due to the action of the environment. An ameiotic form evolving for a long period of time might be expected eventually to lose its diploid character in both the genetic and the cytological sense, its two chromosome sets having become almost completely unlike. Moreover, since no

Plate II

Figure 10. A germinal vesicle of full growth in which the thread-like structures have disappeared and a fine-meshed net has made its appearance. Note the 13 chromosomes: two dot-shaped, the rest all dumb-bell shaped indicating doubleness.

Figure 11. A germinal vesicle beginning to shrink, exhibiting an irregular outline.

Figures 12–17. Germinal vesicles of increasingly reduced size in which the fine-meshed net is in turn replaced by fibers.

Figure 17a. The same germinal vesicle as depicted in Figure 17 but at a lower level of focus, showing signs of disintegration of its membrane.

Figures 18 and 19. Chromosomes lying free in the cytoplasm close to the wall of the oocyte.

Figure 20. Chromosomes have pulled away from the cell wall and become more scattered in an area of material which clearly appears to be more vacuolated than the cytoplasm elsewhere in the egg.

Figure 21. Chromosomes lying in an area of light-staining material in which short sections of fiber can be vaguely seen.

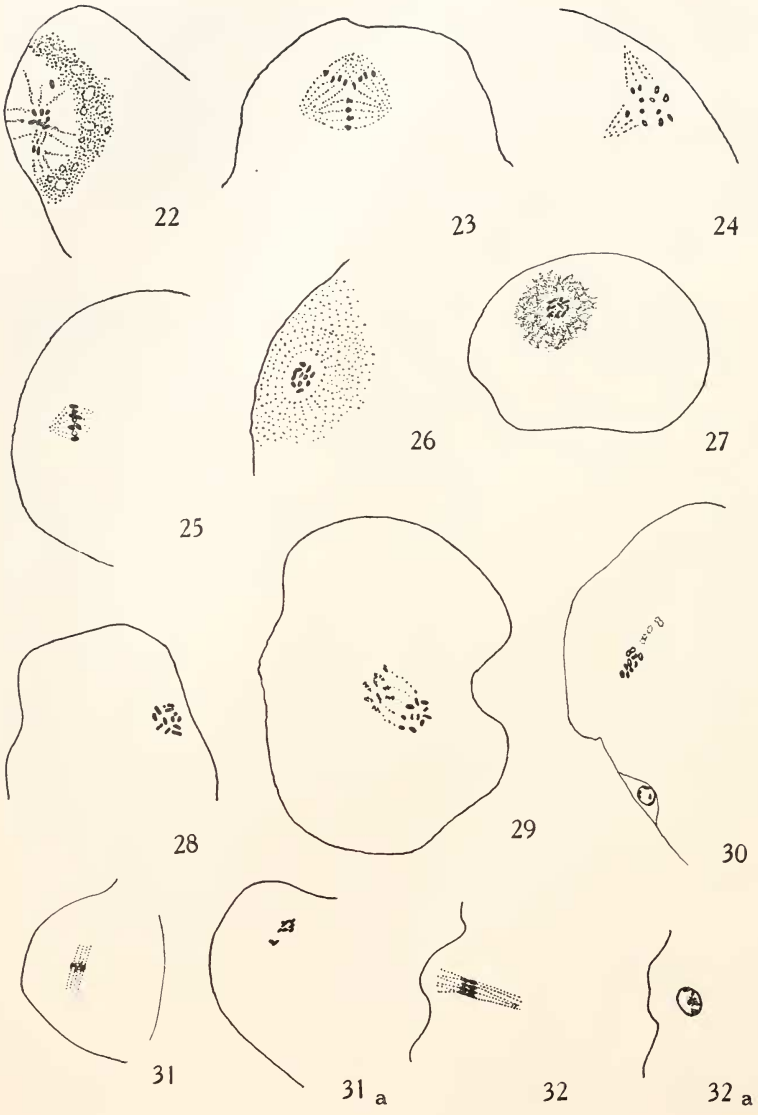


PLATE III

pairing of chromosomes takes place during the maturation of the eggs, there is no 'mechanical' barrier to the establishment of any type of polyploidy in such forms and various forms of aneuploidy, due to irregular reduplication of some chromosome elements, must be expected to occur."

It seems to me that at least three chromosomes in *Philodina roseola* may very well have been involved in structural rearrangement of some kind, though not necessarily just among themselves (Fig. 37). This conclusion should hold unless we assume that the bisexual ancestor of this form had one pair of dimorphic chromosomes and another one without a mate. But this seems to me unlikely. Besides, although reports on chromosome number in rotifers are very confusing, none of them besides the two forms under discussion has been reported to possess an odd number of chromosomes (Makino, 1951) (p. 11). It would seem, then, that the odd number of chromosomes seen in the two species of Bdelloidea under discussion may indicate aneuploidy due either to irregular reduplication of some chromosome elements or some such structural rearrangements as centric fusion accompanied by the loss of one chromosome.

It is difficult to say whether or not the two dot-shaped chromosomes were originally members of the same homologous pair. But since they are present in both *Philodina roseola* and *Habrotrocha tridens,* and since there is no other chromosome that is comparable to them in morphology, it may be safe to look upon them as originally forming a pair. In that event, their disparity in size and the fact that the smaller one of the two, especially in *Philodina roseola,* often stains less intensely than the bigger one could perhaps be regarded as indications of loss of homology between them.

Turning next to the chromosome which in *Philodina roseola* is conspicuously longer than the rest, I must say I cannot confidently identify it in *Habrotrocha tridens.* This is the only morphological difference I can point out with confidence between the chromosome complexes of these two forms.

It should perhaps be stressed here that the absence of pairing of chromosomes in these two forms should not be interpreted necessarily as evidence of loss of homology on the part of their chromosomes, since according to the genetic principle applying to ameiotic parthenogenetic organisms the loss of the diploid character between homologous member chromosomes is possible precisely because of asynapsis.

Plate III

Figure 22. Chromosomes in an area in which the light-staining material is replaced by coarse fibers reaching between the chromosomes and connecting them to the boundary of this area.

Figure 23. A compound spindle consisting of three bipolar spindles, each with its own equatorial chromosome plate.

Figure 24. A compound spindle disintegrating.

Figure 25. Lateral view of a first polar spindle.

Figures 26–28. Polar view of three equatorial plates of the first polar division.

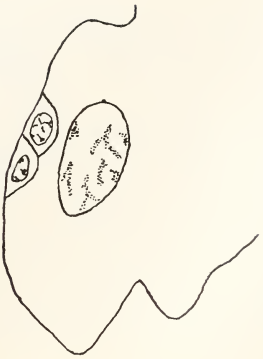
Figure 29. A mitotic figure at anaphase of the first maturation division.

Figure 30. The first polar-body and the chromosomes within the secondary oocyte regrouping to undergo the second maturation division.

Figure 31. A metaphase spindle of the second maturation division.

Figure 31a. Chromosomes belonging to the first polar-body seen at a higher level of focus directly above the metaphase spindle represented in Figure 31.

Figures 32–32a. The same as Figures 31 and 31a except in this case the first polar-body nucleus is already formed.



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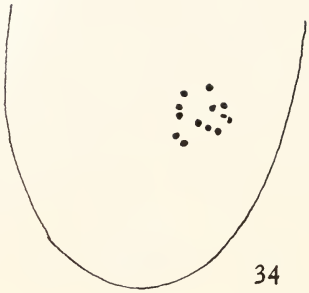

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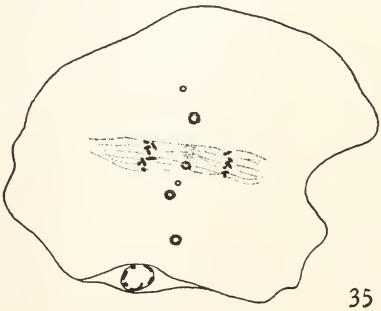

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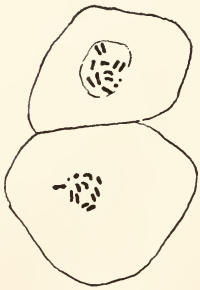

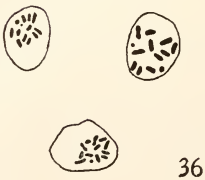

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Plate IV

In other words, asynapsis is here supposed to be antecedent to the loss of homology. Moreover, the persistent condensed state of the chromosomes in my material complicates the situation, since our current theory explaining pairing of chromosomes takes into account, besides the singleness of the threads, also the degree of their uncoiling. In this connection we should, of course, recall that in Neurospora, MacClintock (1945) has reported pairing of relatively contracted chromosomes. Incidentally, it may be mentioned that since daughter chromosomes in these forms can separate without difficulty, the coiling which their chromonemata assume must be of the paranemic type, using the term which Sparrow, Huskins and Wilson (1941) have proposed.

With regard to the first polar spindle, it must be said that due to the paucity of observations the situation in this rotifer is not clear. It is difficult to venture an opinion as to whether or not a tripolar spindle represents a normal stage of development. More observations are needed before a reliable answer can be given. At present, I can only say with confidence that the two spindles shown in Figures 23 and 24 are very distinct and unmistakable structures. Although I have not made an attempt to study the mitochondria condition in *Habrotrocha tridens,* I feel quite certain that mitochondria are not involved in this case, Devisé (1922) and Junger (1931, 1934) notwithstanding. The three separate equatorial plates of chromosomes, one on each spindle, ought to settle the question.

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Summary

1. The pattern of chromosome behavior during egg formation in *Habrotrocha tridens* is the same as that found in *Philodina roseola.* The oocytes undergo two maturation divisions, both equational.

2. The zygoid chromosome number is 13, the same as that of *Philodina roseola.*

3. The pair of dot-shaped chromosomes of unequal size is found in each of these forms, though the conspicuously longer one seen in *Philodina roseola* (Fig. 37) cannot be identified in the present form (Fig. 36).

Plate IV

Figure 33. A portion of a mature egg with a comparatively large nucleus about ready to undergo the first cleavage division. Note the two polar-bodies.

Figure 34. Polar view of the equatorial plate of the first cleavage division. Note the 13 chromosomes, two of which are appreciably smaller than the rest.

Figure 35. An anaphase figure of the first cleavage division. Note the polar-body and the "elimination bodies."

Figure 36. One late prophase and four metaphase chromosome plates seen in the embryonic cells of *Habrotrocha tridens.* Note the two dot-shaped chromosomes.

Figure 37. Two metaphase chromosome plates seen in the embryonic cells of *Philodina roseola.* Note the two dot-shaped chromosomes and the one that is conspicuously longer than the rest.

4. No sign of synapsis has been observed in either form.

5. The chromosomes exist in a condensed condition in the nuclei of the ovary after the last oogonial division, and remain condensed throughout at least the first maturation division.

LITERATURE CITED

Devisé, R., 1922. La figure achromatique et la plaque cellulaire dans les microsporocytes du *Larix europaea*. *La Cellule,* **32**: 249–312.

Hsu, W. S., 1956. Oogenesis in the Bdelloidea rotifer, *Philodina roseola*. *La Cellule,* **57**: 283–296.

Jungers, V., 1931. Figures caryocinétiques et cloisonnement du protoplasme dans l'endosperm d'*Iris pseudo-acorus*. *La Cellule,* **40**: 5–82.

Jungers, V., 1934. Mitochondries, chromosomes et fuseau dans les sporocytes d'*Equisetum limosum*. *La Cellule,* **43**: 321–340.

MacClintock, B., 1945. Neurospora. I. Preliminary observations of the chromosomes of *Neurospora crassa*. *Amer. J. Bot.,* **32**: 671–675.

Makino, S., 1951. Chromosome number in animals. The Iowa State College Press, Ames, Iowa.

Sparrow, A. H., C. L. Huskins and G. B. Wilson, 1941. Studies on the chromosome spiralization cycle in Trillium. *Canad. J. Res.,* **19**: 323–336.

White, M. J. D., 1954. Animal cytology and evolution. The Cambridge University Press.